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BRIEF REPORT

Interaction between variants in the *CYP2C9* and *POR* genes and the risk of sulfonylurea-induced hypoglycaemia: A GoDARTS Study

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Data on the association of *CYP2C9* genetic polymorphisms with sulfonylurea (SU)-induced hypoglycaemia (SH) are inconsistent. Recent studies showed that variants in the P450 oxidoreductase (*POR*) gene could affect *CYP2C9* activity. In this study, we explored the effects of *POR**28 and combined *CYP2C9**2 and *CYP2C9**3 genotypes on SH and the efficacy of SU treatment in type 2 diabetes. A total of 1770 patients were included in the analysis of SU efficacy, assessed as the combined outcome of the HbA1c reduction and the prescribed SU daily dose. Sixty-nine patients with severe SH were compared with 311 control patients. The number of *CYP2C9* deficient alleles was associated with nearly three-fold higher odds of hypoglycaemia (OR, 2.81; 95% CI, 1.30-6.09; *P* = .009) and better response to SU treatment (β , -0.218; SE, 0.074; *P* = .003) only in patients carrying the *POR**1/*1 genotype. Our results indicate that interaction between *CYP2C9* and *POR* genes may be an important determinant of efficacy and severe adverse effects of SU treatment.

KEYWORDS

hypoglycaemia, pharmacogenetics, sulphonylureas, type 2 diabetes

1 | INTRODUCTION

Despite development of novel pharmacological agents, sulfonylureas (SUs) continue to be a cornerstone in type 2 diabetes (T2D) treatment. The most common and potentially most serious adverse effect of sulfonylurea therapy is hypoglycaemia, which limits their use.¹ Severe hypoglycaemia may result in significant morbidity, including higher risk of dementia, stroke and mortality.²⁻⁴ In addition to established clinical factors, identification of genetic factors which can increase the risk of hypoglycaemia can contribute to safer treatment with SU agents.

SUs are metabolized in the liver primarily by the *CYP2C9* enzyme.⁵ *CYP2C9* is highly polymorphic, with *CYP2C9**1 as a major

allele. The 2 most common variants, *CYP2C9**2 (R144C, rs1799853) and *CYP2C9**3 (I359L, rs1057910), have been associated with impaired function and poor metabolism phenotypes⁶ (Table S1). The impact of *2 and *3 polymorphisms on the pharmacokinetics of SUs has been demonstrated in studies in healthy subjects.⁷ In the largest study to date on the effect of *CYP2C9* variants on therapeutic response to SUs, we showed an association of *CYP2C9**2 and *CYP2C9**3 alleles with greater glycaemic response to SUs and a lower rate of treatment failure,⁸ confirming earlier pharmacokinetic data. In line with this, in the Rotterdam study, patients with T2D carrying the *CYP2C9**3 allele required a lower dose of tolbutamide to regulate glycaemia, compared to patients with the wild-type *CYP2C9* genotype.⁹

Despite these findings, limited data from small-sized studies have not provided convincing evidence of the association between CYP2C9 poor-metabolizer genotypes and the risk of SU-induced hypoglycaemia.¹⁰ A small study recently showed that a common polymorphism in the gene encoding a CYP450 redox partner, enzyme P450 oxidoreductase (POR), *POR**28 (A503V, rs1057868), could mask the effect of CYP2C9 variants on the risk of hypoglycaemia associated with SU treatment.¹¹ POR transfers electrons from NADPH to the CYP450 enzymes and is essential for their activity. The common *POR**28 variant showed modest increase in CYP2C9-mediated metabolism of 3 different model substrates in an in vitro study.¹² In line with this, the effect of CYP2C9 reduced-function variants on CYP2C9 activity may manifest only in combination with the *POR**1/*1 genotype.¹¹

In this study, we aimed to assess the impact of CYP2C9*2, CYP2C9*3 and *POR**28 alleles, and their interaction, on SU-induced hypoglycaemia in patients with T2D. Furthermore, we explored the association of the CYP2C9 and *POR* variants with the efficacy of SU treatment in T2D.

2 | METHODS

We used the Scottish Ambulance Service (SAS) data and the Accident and Emergency (A and E) diagnosis records to identify cases of severe hypoglycaemia in Tayside and Fife, Scotland. These data were linked to the Genetics of Diabetes Audit and Research Tayside Study (GoDARTS) database, which contains genetic information and longitudinal biochemistry records, prescriptions and other clinical data in an anonymized form, on nearly 10 000 patients with T2D in Tayside, from 1992 onwards. The GoDARTS study was approved by the Tayside Medical Ethics Committee. Informed consent was obtained for all participants. The use of the GoDARTS bioresource for the study of diabetes pharmacogenetics was approved by the Tayside Tissue Bank.

We identified a total of 339 patients with T2D who were under treatment with SUs, and had experienced a hypoglycaemic event requiring medical assistance in the period 2008 to 2015. Among these, there were 69 patients who were in the GoDARTS study and for whom genetic data were available. Between 1 and 5 controls were selected for each case from the patients with T2D who had experienced no hypoglycaemic events and were treated with SUs during the same time period. These controls were matched for age, sex and age of T2D diagnosis. Patients treated with insulin were excluded. The date of the SAS attendance or A and E admission was taken as an index date for cases and their respective controls.

The pharmacogenetic study of SU response in the GoDARTS study was described previously in detail.⁸ In the current study, we included 1770 patients with T2D who were incident users of SUs during the period 1994 to 2010. SU response was defined as the difference between pre-treatment HbA1c and the minimum HbA1c measured within 1 to 18 months after initiation of SU. Linear regression was used to model the outcome of HbA1c reduction, using pre-treatment HbA1c, age, sex, BMI, adherence, average daily dose, baseline gap (time between pre-treatment HbA1c measurement and

initiation of SU therapy) and a treatment group (SU prescribed as monotherapy or dual therapy: SU added to stable metformin treatment) as covariates.

In addition to HbA1c reduction, we used 2 other outcomes for efficacy, to take into account both HbA1c reduction and dosing of SU as composite measures of response: (1) the average prescribed dose of SU in the treatment period until minimum HbA1c was achieved and (2) a combined outcome of HbA1c reduction and prescribed SU daily dose, assessed as an aggregated Z-score:

$$\text{Combined Z-score} = \frac{\text{dose} - \text{mean}(\text{dose})}{\text{SD}(\text{dose})} - \frac{\Delta\text{HbA1c} - \text{mean}(\Delta\text{HbA1c})}{\text{SD}(\Delta\text{HbA1c})}$$

Thus, a lower combined Z-score indicates a better response to SU treatment. As patients were treated with different SU drugs, the prescribed daily dose was expressed as the percentage of maximum daily dose recommended by the British National Formulary.

The genotypes for CYP2C9*2, CYP2C9*3 and *POR**28 variants were obtained from existing genome-wide data.¹³ The frequencies of the minor alleles of CYP2C9*2, CYP2C9*3 and *POR**28 were 12%, 7% and 27%, respectively. There was no deviation from Hardy-Weinberg equilibrium for any polymorphism. The genotypes for CYP2C9*2 and CYP2C9*3 were combined and analysed as the number of the CYP2C9*2 and CYP2C9*3 reduced-function alleles: 0 (*1/*1), 1 (*1/*2, *1/*3) or 2 (*2/*2, *2/*3, *3/*3), in line with our previous study.⁸ An additive genetic model was used to assess the impact of the *POR**28 variant, and the number of CYP2C9 reduced-function alleles, on hypoglycaemia and SU treatment outcomes.

Conditional logistic regression was used to analyse the effect of the genotypes on hypoglycaemia. The interaction between *POR* and CYP2C9 combined genotypes was assessed by adding an interaction term to the regression model. In the analyses stratified by genotype, matching was broken and an unconditional logistic regression was used. All analyses were adjusted for age, sex, age of T2D diagnosis, BMI, creatinine and HbA1c. Statistical analysis was performed using SAS 9.3 software (SAS Institute Inc., Cary, North Carolina). The statistical significance level was set to $P < .05$.

3 | RESULTS

The characteristics of patients with and without hypoglycaemia are shown in Table S2. Compared to control patients, patients with hypoglycaemia had lower BMI, lower HbA1c and higher creatinine levels. There was no difference in co-treatment with medications known to be CYP2C9 inhibitors.

When assessed individually in the conditional logistic regression model, none of the variants showed association with hypoglycaemia (Table S3). Next, we explored the interaction between combined CYP2C9 and *POR* genotypes by adding an interaction term to the model. The interaction between the 2 genes was significant ($P = .007$). In the analysis stratified by the *POR* genotypes, the number of CYP2C9 deficient alleles increased the odds of hypoglycaemia nearly 3-fold (OR, 2.81; 95% CI, 1.30-6.09; $P = .009$) in the *POR**1/*1 genotype group, whereas there was no effect in the *POR**28

variant carriers (Table 1). However, when stratified by *CYP2C9* genotypes, the minor *POR**28 allele showed no association with hypoglycaemia in either group (Table 1).

We tested the association of genotypes with SU response assessed as 3 different endpoints. The characteristics of patients included in the analysis are presented in Table S4. The effects of variants on the 3 outcomes are shown in Table S5. The *CYP2C9**3 variant (β , 0.145; SE, 0.063; P = .022) and the number of *CYP2C9* reduced-function alleles (β , 0.098; SE, 0.041; P = .017) were associated with better SU response, assessed as HbA1c reduction, in line with our previous study.⁸ No variant showed any effect on prescribed SU dose, whilst *POR**28 showed a marginal association with a lower combined Z-score (β , -0.091; SE, 0.045; P = .043) and thus better response to SU treatment (Table S5).

There was a significant interaction between *CYP2C9* and *POR* genotypes, with both the prescribed dose (P = .004) and the combined Z-score (P = .005) outcomes, but not with the simple reduction in HbA1c outcome (P = .484). In the stratified analysis, the reduced-function *CYP2C9* alleles were associated with a lower combined Z-score (greater response) only in patients with the *POR* *1/*1 genotype (β , -0.218; SE, 0.074; P = .003) (Table 2). On the other hand, the *POR**28 allele showed association with better response only in *CYP2C9**1/*1 carriers (β , -0.167; SE, 0.058; P = .004) (Table 2).

TABLE 1 Association of *CYP2C9* and *POR* genotypes with SU-induced hypoglycaemia: analyses stratified according to *CYP2C9* and *POR* genotypes

Effect of <i>CYP2C9</i> deficient alleles on hypoglycaemia - analysis stratified by <i>POR</i> genotype			
<i>POR</i> genotype	Cases/Controls	OR (95% CI)	<i>P</i>
<i>POR</i> *1/*1	29/151	2.81 (1.30-6.09)	.009
<i>POR</i> *1/*28, *28/*28	33/133	0.70 (0.32-1.57)	.390
Effect of <i>POR</i> *28 minor allele on hypoglycaemia - analysis stratified by <i>CYP2C9</i> genotype			
<i>CYP2C9</i> genotype	Cases/Controls	OR (95% CI)	<i>P</i>
<i>CYP2C9</i> *1/*1	35/180	1.58 (0.82-3.03)	.174
<i>CYP2C9</i> *2 or *3 carriers	27/104	0.58 (0.26-1.28)	.177

TABLE 2 Association of *CYP2C9* and *POR* genotypes with SU response, assessed as the combined outcome of HbA1c reduction and prescribed daily dose (combined Z-score): analyses stratified according to *CYP2C9* and *POR* genotypes

Effect of <i>CYP2C9</i> deficient alleles on combined Z-score - analysis stratified by <i>POR</i> genotype				
<i>POR</i> genotype	N	Beta	SE	<i>P</i>
<i>POR</i> *1/*1	928	-0.218	0.074	.003
<i>POR</i> *1/*28, *28/*28	842	0.035	0.065	.588
Effect of <i>POR</i> *28 minor allele on combined Z-score - analysis stratified by <i>CYP2C9</i> genotype				
<i>CYP2C9</i> genotype	N	Beta	SE	<i>P</i>
<i>CYP2C9</i> *1/*1	1130	-0.167	0.058	.004
<i>CYP2C9</i> *2 or *3 carriers	640	0.059	0.071	.412

4 | CONCLUSIONS

Although earlier studies demonstrated the influence of *CYP2C9* genetic polymorphisms on the metabolism and glycaemic response to SUs, data on the association between *CYP2C9* genotypes and SU-induced hypoglycaemia are limited and inconsistent.¹⁰ The results of our study indicate that these discrepancies could be explained, at least in part, by the interaction between *CYP2C9* and *POR* genes. Although *CYP2C9* deficient alleles were not associated with hypoglycaemia in the entire group of patients, they increased the odds of hypoglycaemic events in patients carrying the *POR**1/*1 genotype. Thus, we replicated a finding from the small study by Ragia et al.¹¹ which showed an association between the *CYP2C9**2 allele and higher risk of hypoglycaemia only in *POR**1/*1 carriers.

Consistent with the effect on hypoglycaemia, the interaction between *CYP2C9* and *POR* genes also showed an association with the efficacy of SU treatment, assessed as a combined outcome of HbA1c reduction and prescribed SU daily dose. The *CYP2C9* deficient alleles were associated with a better treatment effect in *POR**1/*1 carriers. This analysis of SU response in a much larger number of patients corroborates our findings for SU-induced hypoglycaemia, which was explored in a smaller cohort.

In line with a previous study,¹¹ the common *POR**28 variant was not associated with hypoglycaemia in the entire group of patients, nor in subgroups stratified by the *CYP2C9* genotype. On the other hand, it showed an effect on better treatment response in *CYP2C9**1/*1 carriers. This finding is in contrast to prior in vitro work, where *POR**28 showed a modest increase in *CYP2C9* activity, with flurbiprofen, diclofenac and tolbutamide as substrates.¹² Our results, on the contrary, imply that *POR**28 could possibly lead to decreased activity of the wild-type *CYP2C9*, and thus to a higher concentration and an enhanced effect of SUs. Although this effect is possible, as it has been demonstrated that the impact of *POR* variants on CYP activities varies with the CYP isoform and the tested substrate,¹⁴ these intriguing results have not been replicated and have to be considered with caution.

In conclusion, we have shown that interaction between the *CYP2C9* and *POR* genes affects the risk of SU-induced hypoglycaemia and the efficacy of SU treatment. These findings can contribute to unravelling the genetic causes of high inter-individual differences in the efficacy and severe adverse effects of SU therapy, and may lead to safer treatment with these agents.

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Conflict of interest

We declare no conflict of interest.

Author contributions

T. D., K. Z. and E. R. P. designed the study. T. D. and K. Z. analysed the data. T. D., K. Z., L. A. D., G. L., C. N. A. P. and E. R. P. interpreted the data. T. D. and E. R. P. wrote the manuscript. All authors critically assessed and reviewed the manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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